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Influence of Artificial Groundwater Lakes on the Abundance and Activity of Bacteria in Adjacent Subsurface Systems

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With 5 Figures and 1 Table

Key words: Groundwater, aquifer, gravel-pit lakes, bacteria, microbial communities

Abstract

Bacterial abundances and activity, estimated by 4′,6-diamidino-2-phenylindole staining (DAPI) and the reduction of 2-(p-iodophenyl) -3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT), were investigated in two oligotrophic artificial groundwater lakes and the surrounding aquifers. To evaluate the effect of lake water on groundwater downstream, samples were taken from wells at different distances from the lakes, and the total number of bacteria and the number of active bacteria in these samples were compared with samples collected upstream. In addition, sterilized sandy sediments were exposed in groundwater wells to measure the number and activity of bacteria attached to particles. At one of the study sites, where the lake sediments were disturbed by dredging, total bacterial abundance and the number of respiring bacteria in the groundwater aquifer was clearly influenced by the lake water. The average bacterial abundances decreased from $2.6 \pm 1.9 \times 10^5$ cells ml^{-1} in the well closest to the lake (S2) to $2.9 \pm 3.8 \times 10^4$ cells ml^{-1} in the most distant one (S4), which was equivalent to cell numbers in the upstream well. The number of respiring bacteria showed a similar tendency with $1.3 \pm 2.7 \times 10^4$ active cells ml^{-1} in S2 and $1.9 \pm 1.5 \times 10^3$ active cells ml^{-1} in S4. At the second study site, which was not influenced by dredging, bacteria in the downstream wells seemed not to be affected by the lake water. The number and activity of bacteria, which colonized exposed sediments, were not significantly different in the upstream and downstream wells, indicating a minor influence of lake water on this habitat. Our results suggest that gravel-pit lakes may influence the free living bacterial assemblages in nearshore groundwater systems, but do not visibly affect numbers and activity of bacteria attached to the surface of aquifer sediments.

Introduction

Over the past decades many lakes have been created by gravel digging activities and subsequent influx of groundwater. The chemical composition of these lakes differs from the surrounding groundwater soon after the exposure of the groundwater to surface conditions, especially as a consequence of shifts in the redox situation and biological processes, mainly primary production. Physico-chemical changes in the gravel-pit may influence the groundwater in the subsurface outflow of the lake, which, apart from its ecological significance, is also of interest for the utilization of water for human use (VANEK 1987; SAMPL et al. 1995).

Most studies of the interactions of surface water and the surrounding groundwater focus on the interaction of streams with groundwater in alluvial systems (WINTER 1995). Although many investigations describe lakes and their relations to adjacent groundwater, for instance in connection with studies on eutrophication or acid precipitation, most studies have been focused on the analysis of hydrological, physical and chemical parameters (LEE 1996; LA BAUGH 1986). Microbiological investigations are rare and cover mainly hygienic aspects (RITTER 1980).

Unpolluted shallow aquifer sediments are usually oligotrophic systems, and indigenous groundwater bacteria depend on the characteristics of the water percolating down from the soil above (BENGTSON 1989). On the other hand, as discussed above, microbial communities in groundwater systems can also be influenced by water entering laterally from surface waters. Therefore physico-chemical characteristics should not be used as the only tools to investigate the interaction of the surface water and the groundwater, because these parameters do not assess induced changes of the autochthonous microbial community in the groundwater system, or the possible transport of microbial cells to the downstream aquifer. Especially in the case that pollution takes place in a lake, it is of major interest to determine the extend of interaction between the lake and the adjacent groundwater based on microbiological investigations.

The aim of this study was to investigate the microbial interactions of gravel-pit lakes with the surrounding shallow groundwater with the specific objectives (1) to examine numbers and activity of free living groundwater bacteria upstream of the lake and from samples taken downstream at different distances from the gravel-pit lake, (2) to determine the effect of lake water entering the downstream aquifer on bacteria attached to particles (i.e. native bacteria) and (3) to assess the potential of the transport of bacteria from the lake to the downstream aquifer. In addition to epifluorescent microscopic direct counts to determine total cell numbers, we investigated the bacterial respiratory activity in single cells to obtain real-time information on the physiological status of the microbial communities *in situ*.

Materials and Methods

Study sites and sampling

The study sites are located near the cities of Klagenfurt (Baggersee Weizelsdorf) and Graz (Schwarzlseen) in the southern part of Austria. Water samples for chemical analysis and for the determination of bacterial numbers and activity were collected monthly from May 1992 to April 1993. In the investigation area at the surrounding of the gravel-pit lake "Baggersee Weizelsdorf", one sampling well (W1) was situated upstream whereas the groundwater wells W2, W3, W4 and W5 were situated downstream at different distances from the lake and influenced by the outflowing lake water (Table 1). At the second sampling site „Schwarzlseen" well S1 was located upstream. Environmental isotope studies suggest that nearshore groundwater wells S2, S3 and the more distant well S4 were influenced hydrologically by outflowing water from the gravel pit (SAMPL et al. 1995; YEDHEGHO 1993).

Since the lake Weizelsdorf was not disturbed by dredging, the lake sediments of Schwarzlseen were partly removed for gravel production during winter time. Details of the investigation area, sampling methods and chemical analysis are published elsewhere (SAMPL et al. 1995).

Prior to sampling, the wells were flushed by pumping at least five well volumes of water until pH and conductivity were stable. For the estimation of bacterial numbers, samples of 100 ml were fixed with formaldehyde solution (2% final conc.). To measure bacterial activity, well water was filled into sterile one liter bottles, kept at ambient temperatures and processed immediately after sampling.

For the preparation of artificial sandy deposits, sediments from the sampling sites were size fractioned, and sediments between 250 and 500 µm particle size were washed twice with distilled water, sterilized by autoclave and dried at 100 °C. These sterile sands were exposed in containers of stainless steel nets with a volume of ca. 400 cm^2 (ALFREIDER et al. 1997). The inner sides of the containers were covered with a 125 µm net. The containers were attached to a string and immersed into the well water approximately 2 m beneath the water table. After 2 months, when the initially sterile sediments were expected to be in a balanced state of microbial colonization (MARXSEN 1982), the containers were transferred into sterile 1 l glass beakers, transported to the laboratory in a refrigerator and processed immediately.

Samples from lake water of Schwarzlseen were collected with a 5 l Schindler-Patalas sampler at 3 m depth. Pelagic water samples of the gravel-pit lake Weizelsdorf were taken by pumping water from 3 m depth.

Determination of bacterial abundances

For determination of total bacterial numbers in water samples, 5 to 20 ml were stained for 10 min with the fluorochrome 4´,6-diamidino-2-phenylindole (DAPI, final conc. 1µg ml^{-1}) according to PORTER & FEIG (1980). Samples were filtered onto black membrane filters (Poretics; 0.2 µm pore size). At least 400 bacteria were counted at 1600x magnification using an epifluorescence microscope (Zeiss Axioplan) equipped with a filterset for UV-excitation (BP 365/ FT 395/LP 397).

Determination of respiring bacteria

Actively respiring bacteria were enumerated by incubation with 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) modified from ZIMMERMANN et al. (1978).

To determine the number of respiring cells, 20 to 50 ml of well water samples and 10 ml of lake water samples were incubated with INT (200 mg l^{-1} final conc.) for 1 hour at *in situ* temperatures. The reaction was stopped with formaldehyde solution (4% final concentration). INT samples were additionally stained with DAPI (final conc. 1 µg ml^{-1}) for 10 minutes. Samples were filtered onto 0.2 µm cellulose nitrate filters. Filters were placed on a glass slide with a drop of glycerine oil on the filter and covered with a cover slip. First, DAPI stained bacteria were counted at a magnification of 1600x as described above, then enumeration of all active bacteria was carried out in the same microscopic field with brightfield microscopy.

Methodological details for the determination of bacterial numbers and activity from sediments are described elsewhere (ALFREIDER et al. 1997).

Results

Chemical and physical characteristics

At the study site Weizelsdorf the concentrations of total organic carbon (TOC), total phosphorus (P-tot) and nitrate (NO_3-N) were very low, and nutrient concentrations measured in groundwater samples were within the range found in lake water (Table 1). Both groundwater and lake water samples of the site Schwarzlseen showed larger concentrations of P-tot and NO_3-N than samples from Weizelsdorf, and the chemical composition of lake water and groundwater samples were of similar magnitude, with exception of groundwater well S1, situated upstream. Nitrate concentrations of about 10 mg l^{-1} in this well were two times higher than concentrations of nitrate in lake water and in the groundwater downstream. Within the groundwater systems, dissolved O_2-concentrations in nearshore wells downstream of the lake were variable and usually lower than in other groundwater wells (Table 1).

Well water upstream from the lake (S1, W1) and in more distant wells downstream (S4, W5) showed little seasonal temperature variations, and values ranged between 5.2 and 12 °C (Fig. 1). In contrast, we found distinct seasonal temperature differences in nearshore groundwater samples and the lakes. The annual range of temperature in nearshore wells

Table 1. Site characteristics of lake- and well water samples. S1 and W1 are situated in the upstream direction, all others downstream. (Med – Median values).

Sampling station		Distance from lake (m)	pH	O_2 (mg l^{-1})	TOC (mg l^{-1})	P-tot (µg l^{-1})	NO_3-N (mg l^{-1})
Schwarzlseen							
	Min		7.1	8.5	1.5	3	8.3
S1	**Med**	Upstream	**7.2**	**8.7**	**1.8**	**8**	**10.7**
	Max		7.3	9.5	2.5	18	12.3
	Min		8	8.2	2.1	6	4.2
Lake	**Med**		**8.2**	**10.9**	**2.5**	**10**	**4.9**
	Max		8.3	14	3	27	5.7
	Min		7.5	1.4	1.2	1	3.4
S2	**Med**	**4**	**7.7**	**5.9**	**1.6**	**8**	**4.7**
	Max		8	11.7	2.1	19	5.9
	Min		7.4	3.6	1.1	3	3.6
S3	**Med**	**20**	**7.5**	**6.5**	**1.9**	**10**	**4.3**
	Max		7.6	8.3	2.9	35	4.7
	Min		7.2	6.7	1.2	6	3.4
S4	**Med**	**1250**	**7.3**	**7.1**	**1.6**	**19**	**4.2**
	Max		7.4	7.6	2.4	99	4.7
Weizelsdorf							
	Min		7.1	8.0	0.9	2	1.3
W1	**Med**	Upstream	**7.5**	**8.8**	**1.1**	**3**	**2.0**
	Max		8.0	9.5	1.4	11	3.8
	Min		7.7	7.5	1.1	3	0.5
Lake	**Med**		**8**	**10.2**	**2.2**	**4**	**1.3**
	Max		8.3	12.2	3.4	6	1.8
	Min		7.1	0.7	0.8	1	0.2
W2	**Med**	**8**	**7.7**	**5.9**	**1.3**	**3**	**1.2**
	Max		8	10.8	2.3	9	1.7
	Min		7.1	2.3	1.1	1	0.8
W3	**Med**	**10**	**7.7**	**6.1**	**2.0**	**2**	**1.1**
	Max		8.0	9.7	3.1	3	1.6
	Min		7.2	2.7	0.9	1	0.7
W4	**Med**	**12**	**7.8**	**7.1**	**1.5**	**2**	**1.4**
	Max		8.2	11.4	2.5	4	2
	Min		7	7.3	0.9	2	1.4
W5	**Med**	**240**	**7.6**	**8.5**	**1.4**	**2**	**2.3**
	Max		8.1	10.1	2.4	4	3.9

W2 and S2 showed similar patterns as the gravel-pit lakes Weizelsdorf and Schwarzlseen (Fig. 1). With increasing distance from the gravel-pit lakes the seasonal variability of groundwater temperature decreased and these seasonal variations in temperature reflect to a certain extend the influence of lake water on groundwater in the downstream direction.

Bacterial abundance

Mean annual total numbers of bacteria in groundwater samples upstream of the gravel-pit lakes were constantly low in both investigation areas. In the gravel-pit lakes, a pronounced increase in bacterial numbers by almost 2 orders of magnitude occurred. Bacterial numbers in groundwater wells downstream the gravel-pit lake Weizelsdorf dropped rapidly to values found upstream in W1 (Fig. 2). Bacterial abundances detected in the nearshore well W2 , about 8 m downstream from the lake, were not much different from W5, situated 240 m apart and less than in the wells W3 and W4 (Fig. 2).

At the study site Schwarzlseen bacterial abundances showed a distance dependent pattern (Fig. 2) similar to the

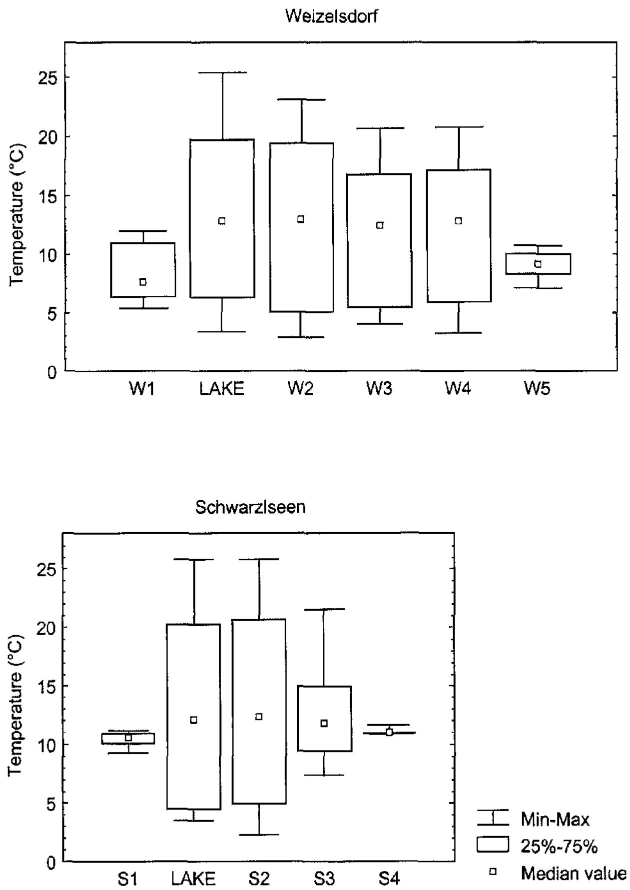

Fig. 1. Annual variations of temperature in different groundwater wells and lake water at the study sites Weizelsdorf and Schwarzlseen.

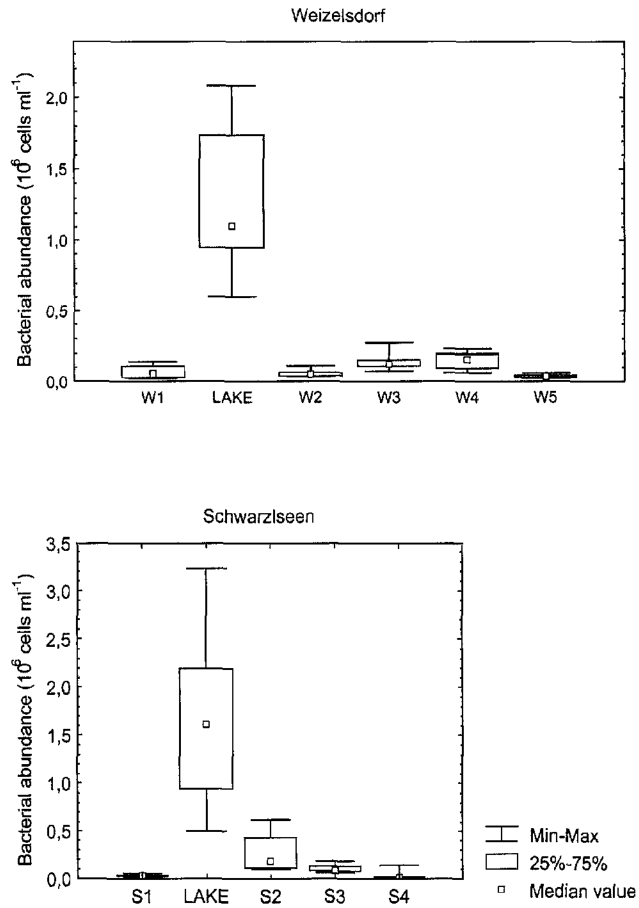

Fig. 2. Annual variations of bacterial abundances in different groundwater wells and lake water at the study sites Weizelsdorf and Schwarzlseen.

variations of the temperature patterns (Fig. 1): With increasing distance from the lake, abundance and seasonal variability significantly decreased in S2 (4 m downstream) and S3 (20 m downstream). Bacterial numbers in S4 were comparable to those found upstream in S1 (Fig. 2).

Abundance and fraction of active bacteria

The annual distribution of the abundance of active bacteria corresponded with the pattern observed for total cell numbers (Fig. 3). We found low numbers of active cells in the upstream well W1 and also in S1, both sampling stations showing minimal seasonal variations. The increase of active bacteria in lake water of Weizelsdorf and Schwarzlseen was comparable to the increase in total cell counts (Fig. 2). The spatial pattern of variability of active bacteria in the groundwater wells at the site Schwarzlseen was characterized by high variations in the well S2 and a decreasing annual variability of active cell numbers from S3, compared to well S4, which was characterized by very low numbers of active bacteria (Fig. 3). The pooled seasonal data of active bacteria downstream of lake Weizelsdorf were less variable than the numbers from the Schwarzlseen site and similar for all groundwater wells (Fig. 3).

In contrast to the number of active bacteria, the fraction of active bacteria in the lake water was not significantly higher than in the groundwater samples (Fig. 4). At the site Schwarzlseen, the fraction of active bacteria in the wells S1 and S4 was, although not statistically different, on average even higher than values in lake water samples. The distribution of fractions of active bacteria at the site Weizelsdorf was more complex, and varied considerably between sampling stations (Fig. 4). At this location no distinct pattern or trend was found. At both sites, however, lowest values appeared in February.

Sediment samples

After exposing sediments in the groundwater wells S1, S3, W1 and W3 for two months, a dense microbial flora had developed. The abundance of bacteria, the number of active bacteria and [^{3}H]thymidine and [^{14}C]leucine incorporation were variable over time (ALFREIDER et al. 1997), but when comparing the annual average (n = 6) of the parameters, no

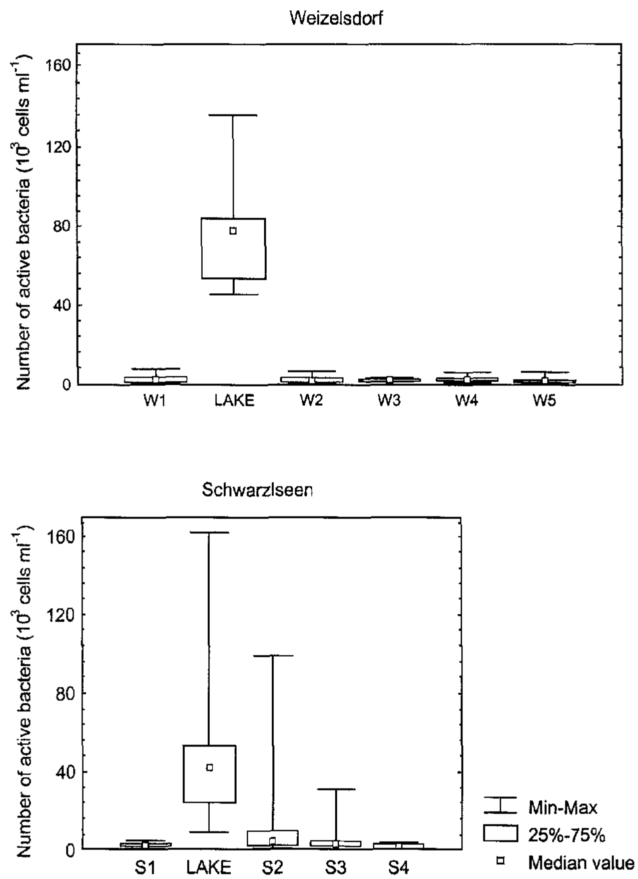

Fig. 3. Annual variations of the number of active bacteria in different groundwater wells and lake water at the study sites Weizelsdorf and Schwarzlseen.

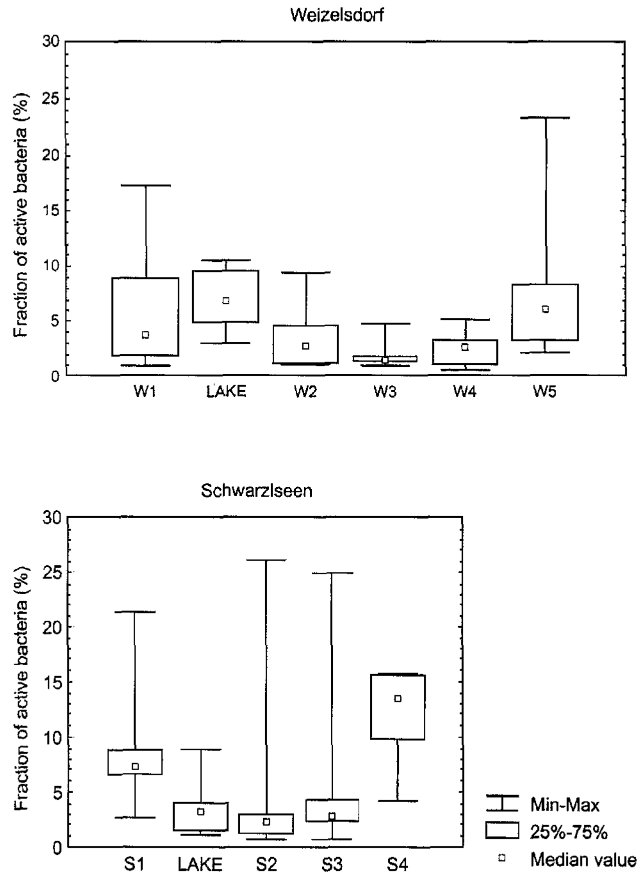

Fig. 4. Annual variations of the fraction of active bacteria in different groundwater wells and lake water at the study sites Weizelsdorf and Schwarzlseen.

significant differences between upstream and downstream groundwater wells were observed (Fig. 5). Furthermore, a comparison between the sites Weizelsdorf and Schwarzlseen indicated that all measured bacterial parameters in the exposed sediments were of similar size at both locations.

Discussion

It is most likely from chemical data of the wells S2 - S4 and W2 - W5 (Table 1) that the outflowing water from the gravel-pit lakes did not create a distance dependent gradient and thus autochthonous bacteria were confronted with comparable chemical conditions at all downstream wells. Seasonal temperature variations caused by the artificial lakes could have exerted some effect on the microbial communities within the first 20 m downstream (Table 1; Fig. 1), and bacterial assemblages in nearshore wells downstream at the site Schwarzlseen were clearly influenced by the lake water. On the other hand, wells downstream of lake Weizelsdorf showed similar temperature patterns, but no distance dependent gradient of bacterial parameters was observed. For this reason, we assume that these differences at nearshore wells may also be caused by the transport of bacteria from the lake into the groundwater system. In this case results suggest that the Weizelsdorf area represents a closed system for bacteria, whereas pelagic bacteria from Schwarzlseen may enter the subsurface aquifer and move along very short distances. It seems that dredging at the study site Schwarzlseen increased the permeability of the lake sediments for pelagic bacteria.

Travel distances of bacteria in groundwater aquifers are difficult to evaluate and often based on predictions from filtration theories or column experiments with pure cultures. Therefore we compared our investigations from the site Schwarzlseen with a field study of Bales et al. (1995), who injected stained bacteria into a sandy aquifer to perform transport studies. They found about 1.7% of the bacteria (input value) 6 m downgradient from the point of injection. They conclude that bacteria could persist over tens of meters downgradient, which corresponds with our findings from the nearshore wells downstream of Schwarzlseen. Bacterial numbers in the well S2, situated 4 m downstream, showed 16.4% of the lake-water abundances and decreased further to 6.6% in S3 (located 20 m from lake shore).

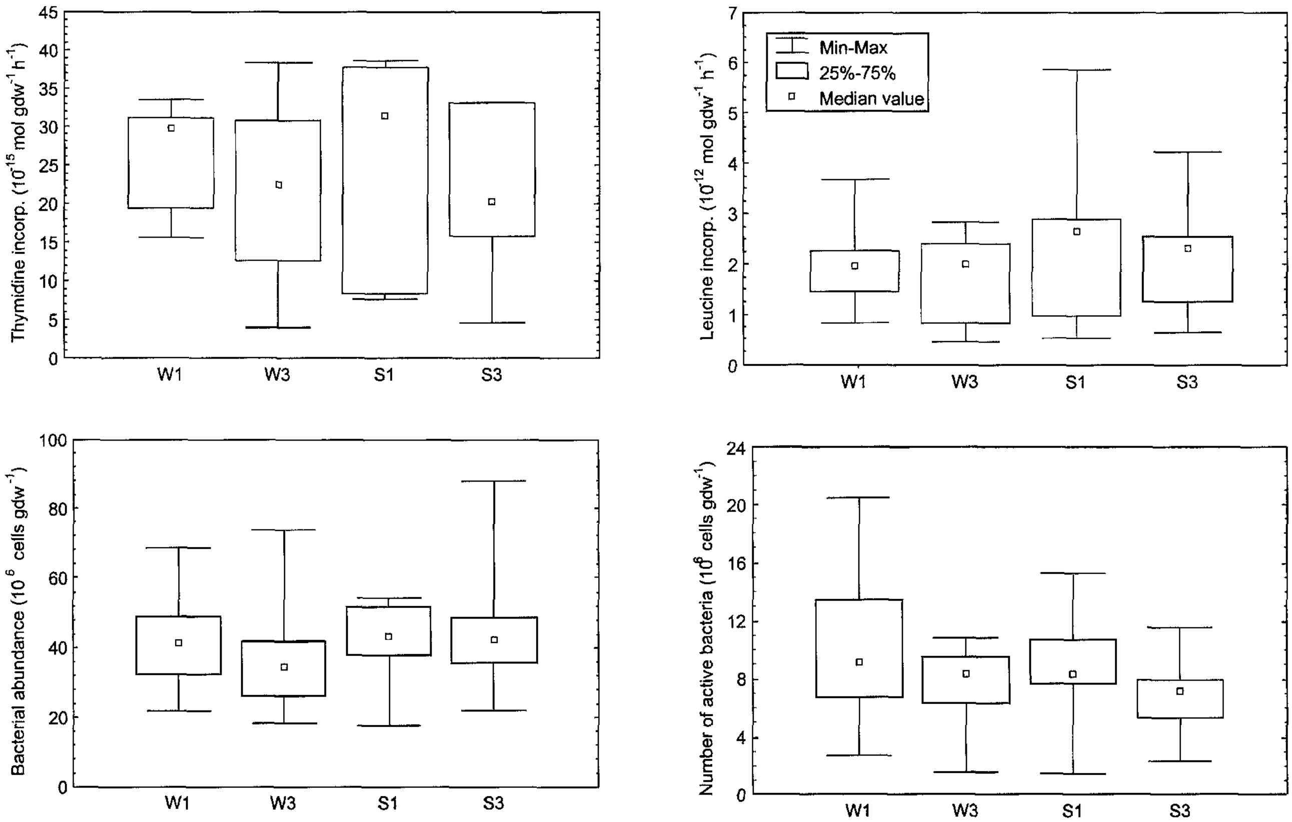

Fig. 5. Annual variations of [^{3}H]thymidine and [^{14}C]leucine incorporation rates, bacterial abundances and numbers of active bacteria in exposed sediments at the study sites Weizelsdorf (W1, W3) and Schwarzlseen (S1, S3).

The assumption that bacteria at the closely related groundwater systems were influenced by the lake at the study area Schwarzlseen is also supported by the comparison of the fraction of active bacteria (Fig. 4). While in the lake water and the closely situated wells S2 and S3 the fraction of active bacteria was on average below 5%, the fraction of active bacteria in the non influenced wells S1 and S4 were much higher, with an annual average of about 12% and 8%, respectively. The data indicate that along a distance gradient from the lake total cell numbers and abundances of active bacteria decreased by 1 or 2 orders of magnitude whereas the fraction of active bacteria increased. It seems that autochthonous groundwater microbial communities replace allochthonous bacteria introduced from the lake into the subsurface system already within a short distance, most probably because lake-water bacteria are not adapted to the environmental conditions in this habitat.

Spatial variations of bacteria in the groundwater of the study site Weizelsdorf were more complex and bacterial parameters in the individual sampling stations showed no distinct patterns from potential lake - groundwater interactions.

Very little is known about the transport of bacteria through geologic media and the main informations are largely based on laboratory studies using artificial models (Gounot 1994). Allochthonous bacteria are usually be transported along short distances in nonfissured aquifers (Gerba & Bitton 1984). They are usually eliminated by biological, chemical and physical processes, and their number decreases exponentially with time (Matthess 1990; Matthess et al. 1989).

The influence of lake water on the groundwater system at the study site Schwarzlseen was only evident in samples from pumped groundwater samples. Investigations of exposed sediment samples suggest that bacteria attached to sediment particles were not affected by the lake (Fig. 5). Autochthonous microorganisms in groundwater systems are usually attached to sediment particles (Gounot 1994) and taking into account that we found - on average - 660 times more bacteria in the sediment samples than in corresponding groundwater sample of the same volume (Alfreider et al. 1997), it is obvious that free living bacteria are just a minor fraction of the microbial assemblages in oligotrophic aquifers (Hazen et al. 1991).

Our results indicate that keeping gravel-pit lakes open to groundwater systems has some effect on the abundance and activity of the free-living bacterial communities in the aquifer at close range and virtually none on the attached groundwater microflora. However, this assumption is probably only valid in the context of aquatic systems characterized by low nutrient concentrations. In shallow and nutrient-rich aquifers differences in abundance and activity between free-living and sediment-attached bacterial assemblages are much less pronounced (HARVEY & GEORGE 1987; HARVEY et al. 1984), and the fate of bacteria transported from surface waters into this type subsurface systems could be quite different. In addition, also other factors, e. g. the sediment texture, are important for the occurrence and activity of bacteria in groundwater systems (ALBRECHTSEN & WINDING 1992).

More research is required to investigate the spatial variations of groundwater bacteria in systems of different trophic states. Furthermore, there is an urgent need for more precise methods that allow us to study the origin and the displacement of bacteria in groundwater systems. The introduction of nucleic acid-based techniques in microbial ecology (JIMENEZ et al. 1991; AMANN et al. 1997) is one application with good prospects.

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